ASED http://e-ASED.org
Animal Systematics, Evolution and Diversity

Anim. Syst. Evol. Divers. Special Issue, No. 9: 1-9, December 2016
https://doi.org/10.5635/ASED.2016.SIN9.041

First Record of Two *Spirostomum* Species (Spirostomatidae, Heterotrichida, Heterotrichea) of Ciliates from Jindo Island in Korea

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ABSTRACT

Two *Spirostomum* species were collected from freshwater in Jindo Island, Korea and identified as *Spirostomum ambiguum* (Müller, 1786) Ehrenberg, 1835 and *Spirostomum minus* Roux, 1901. This study is the first known morphological record of these two species being found in Korea. The description is based on the observation of living specimens and protargol impregnated specimens. Diagnostics of *Spirostomum ambiguum*: body size 340−930 × 45−80 μm *in vivo*; long and slender body with truncated posterior part; macronucleus moniliform with 7−22 nodules; cortical granules irregularly arranged 4−5 rows in between somatic kineties; 24−58 somatic kineties arranged longitudinally; adoral zone of membranelles (AZM) covered about 60−80% of body length. Diagnostics of *Spirostomum minus*: body size 500−730 × 35−45 μm *in vivo*; long and slender body with truncated posterior part; macronucleus moniliform with 11−16 nodules; micronucleus 20−37 oval shape; cortical granules regularly arranged 3−4 rows in between somatic kineties; 20−30 somatic kineties arranged longitudinally; AZM covered about 40−50% of body length with 120−150 adoral membranelles.

Keywords: freshwater, morphology, redescription

INTRODUCTION

The family Spirostomidae is characterized by a medium to large size, cylindrical body shape and very contractile body, holotrichous somatic ciliation, peristomial field covering 50−75% of body length, and posterior location of contractile vacuole. Four genera have been recognized, including two genera *incertae sedis* in the family Spirostomidae (Lynn, 2008). However, genus *Gruberia* was excluded and genus *Anigstenia* included recently from family Spirostomidae (Shazib et al., 2014). The genus *Spirostomum* was differentially diagnosed genus *Trichoda* by a long collecting canal from the contractile vacuole to the anterior part, a truncated posterior end and a long buccal field on the body edge (Ehrenberg, 1833, 1838; Curds et al., 1983; Jang et al., 2012). The *Spirostomum* has been known eight species worldwide, and two species of them have been recorded morphologically in Korea up to now (Claparède and Lachmann, 1858; Kahl, 1932; Shigenaka, 1959; Dragesco and Dragesco-Kernéis, 1986; Foissner et al., 1992; Jang et al., 2012; Boscaro et al., 2014). In this study, we describe two species from Jindo Island, Korea.

MATERIALS AND METHODS

Sample collection and culture

Spirostomum ambiguum was collected from the Bongam-reservoir in Gachi-ri, Jisan-myeon, Jindo-gun, Jeollanam-do (34°24′58.3″N, 126°07′44.2″E), Korea on 8 Jul 2016. *Spirostomum minus* was collected from a freshwater pond in the temple Cheonjong-sa, Simdong-ri, Jisan-myeon, Jindo-gun, Jeollanam-do (34°24′54.8″N, 126°07′27.5″E), Korea on 8 Jul 2016. The surface water (~30 cm) including debris were collected and transferred to Petri dishes with sediments then maintained in the laboratory for several days at room temperature. A few wheat grains were added to the raw culture of Petri dishes for the enrichment of bacteria and ciliates (Li et al., 2010).

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pISSN 2234-6953 eISSN 2234-8190

Morphological observation

Cells were observed under a dissecting microscope using low magnification and an optical microscope at high magnification. The infraciliature was revealed by protargol impregnations (Wilbert, 1975). Drawings of living cells were based on free-hand sketches. Classification and terminology are based on Lynn (2008).

SYSTEMATIC ACCOUNTS

Phylum Ciliophora Doflein, 1901
Subphylum Postciliodesmatophora Gerassimova and Servin, 1976
Class Heterotrichea Stein, 1859
Order Heterotrichida Stein, 1859
Family Spirostomidae Stein, 1867
Genus *Spirostomum* Ehrenberg, 1833

***Spirostomum ambiguum* (Müller, 1786) Ehrenberg, 1835 (Tables 1, 2, Figs. 1–3)**

Trichoda ambigua Müller, 1786: 200.

Spirostomum ambiguum: Ehrenberg, 1835: 165; 1838: 332; Stein, 1867: 197; Kahl, 1932: 437; Boggs, 1965: 605; Repak & Isquith, 1974: 326; Dragesco & Dragesco-Kernéis, 1986: 374; Foissner et al., 1992: 317; Boscaro et al., 2014: 527.

Diagnosis. Body size on average 660 × 60 μm *in vivo*; long and slender body with truncated posterior end; macronuclei moniliform with 7–22 nodules; cortical granules regularly arranged 4–5 rows in between somatic kineties; 24–58 somatic kineties arranged longitudinally; adoral zone of membranelles covered about 60–80% of body length.

Description. Body size 340–930 × 45–80 μm *in vivo*, approximately 660 × 60 μm. Body flexible, long and slender with rounded anterior and truncated posterior ends, body length to width ratio approximately 11 : 1 (Figs. 1A, 2A), and fusiform body when contracted (Figs. 1B, C, 2B, 3A). Macronuclei moniliform with 7–22 nodules (Figs. 1A, B, 2C, 3C), nodule size about 35 × 20 μm in live specimens (Figs. 1A, 2C). Contractile vacuole located posterior end, occupied approximately 1/6–1/16 of body length with one long canal that extended to anterior end of dorsal side (Figs. 1A, 2D, E). Cytoplasm colored brownish. Cortical granules irregularly arranged in 4–5 rows between somatic kineties, yellowish, approximately 0.5 μm in diameter *in vivo* (Fig. 2F). Movement relatively slow, usually spirally swimming around its body axis. Somatic kineties longitudinally arranged and 24–58 in number, commenced along the apical end to left side of the adoral zone of membranelles and spirally curved when contractiled (Figs. 1A, B, 2B, 3A), Adoral zone of membranelles occupied 60-80% of body length *in vivo* (Figs. 1A, 2A). Cytostome located at proximal end of adoral zone of membranelles, crescent shaped (Figs. 1A, B, 2E, G, 3A, D).

Table 1. Morphometrical characterization of *Spirostomum ambiguum* (A) and *Spirostomum minus* (M)

Characters (*in vivo*)	Species	Mean	Med	Min	Max	SD	SE	CV	n
Body length (μm)	A	658	660	338	930	119.76	27.48	18.19	19
	M	627	624	500	734	57.39	13.17	9.14	19
Body, width (μm)	A	62	61	47	82	8.34	1.91	13.39	19
	M	43	43	36	47	2.53	0.58	5.84	19
Body length/Body width, ratio	A	10.65	10.22	7.16	14.27	2.02	0.46	18.97	19
	M	14.48	14.38	12.67	15.96	1.10	0.23	6.94	19
MA, number	A	15	16	7	22	4.31	1.02	27.74	19
	M	14	14	11	16	1.57	0.36	11.44	19
MA, length	A	34	32	11	67	12.71	2.92	37.49	19
	M	25	25	19	35	3.87	0.89	15.27	19
MI, number	M	27	27	20	37	4.02	0.92	14.79	19
Somatic kineties, number	A	44	46	24	58	10.30	2.57	23.54	16
	M	26	26	20	30	1.29	0.30	9.82	19
Contractile vacuole/Body length, ratio (%)	A	12	12	6	17	2.66	0.61	22.03	19
	M	18	18	8	25	3.84	0.88	20.99	19
Cortical granules, number of rows between somatic kineties	A	4	4	4	5	0.51	0.14	11.60	15
	M	4	4	3	4	0.51	0.12	14.17	19
Cortical granules, diameter	A	0.47	0.5	0.3	0.6	0.09	0.02	18.22	13
	M	0.39	0.4	0.3	0.5	0.06	0.01	14.10	17
AZM length/Body length, ratio (%)	A	69	67	59	79	6.03	1.91	8.90	19
	M	46	46	38	51	3.48	0.80	7.61	19
Adoral membranelles, number	M	135	136	120	150	7.99	1.83	5.92	19

Mean, arithmetic mean; Med, median value; Min, minimum; Max, maximum; SD, standard deviation, SE, standard error, CV, coefficient of variation in %; MA, macronucleus; MI, micronucleus; AZM, adoral zone of membranelles.

Table 2. Comparisons of *Spirostomum* congeners

Characters	*S. ambiguum*	*S. ambiguum*	*S. ambiguum*	*S. minus*	*S. minus*	*S. minus*	*S. minus*
Body length (L) (*in vivo*, μm)	340–930	900–thousands	1,000–4,000	500–730	350–900	400–800	300–800
Body width (W) (*in vivo*, μm)	45–80	–	48–100	35–45	–	55–115	30–40
Body length/Body width, ratio (%)	11 : 1	9–17 : 1	10–17 : 1	14 : 1	7–15 : 1	–	10–20 : 1
AZM length/Body length, ratio (%)	60–80	50–66	65–70	40–50	50	50	35–50
Adoral membranelles, number	–	–	–	120–150	–	–	–
Contractile vacuole/ Body length, ratio (%)	6–17	Up to 10	–	8–25	20	–	–
Macronucleus, number	7–22	12–50	10–50	11–16	5–25	9–25	8–50
Micronuclei, number	–	100>	–	20–37	Up to 20	3–30	–
Somatic kineties, number	24–58	30–50	70–90 (contractile status)	20–30	12–24	30–40	20–30
Number of CG rows[a]	4–5	4–5	4–5	3–4	2–4	–	3–4
CG, color	Yellowish	–	Yellowish	Colorless	–	Pale brown	–
Data source	Present study	Boscaro et al. (2014)	Foissner et al. (1992)	Present study	Boscaro et al. (2014)	Fernandes and da Silva Neto (2013)	Foissner et al. (1992)

AZM, adoral zone of membranelles; CG, cortical granules; –, data unavailable.
[a]Number of cortical granular rows in between somatic kineties.

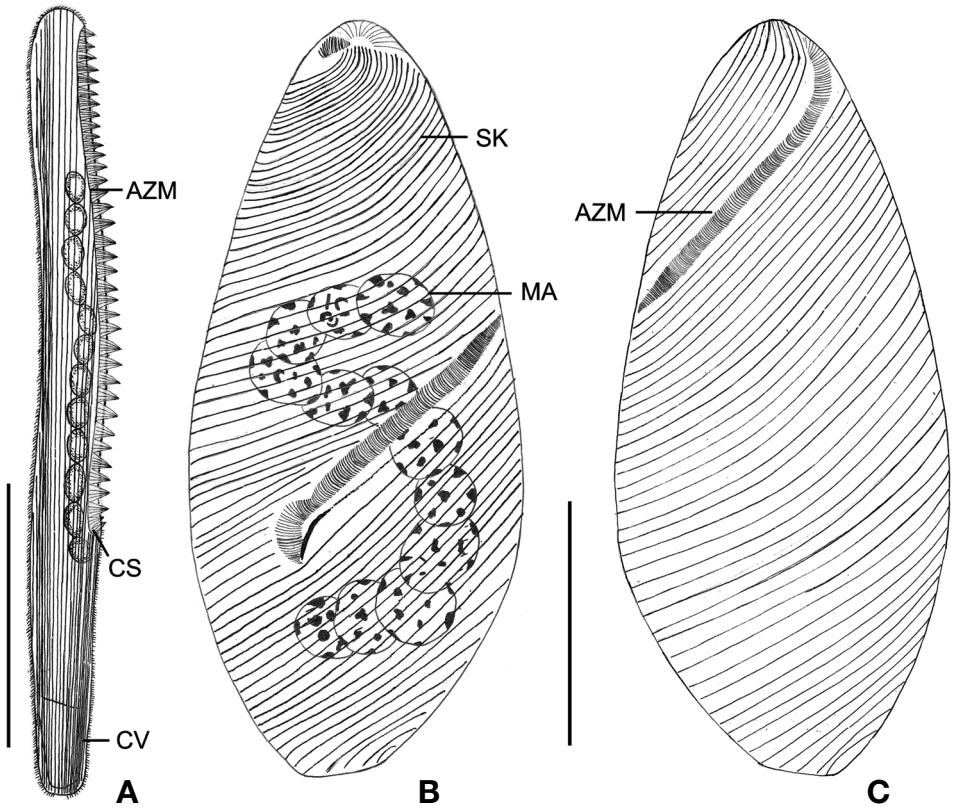

Fig. 1. *Spirostomum ambiguum* from a live specimen (A) and after protargol impregnation (B, C). A, Right side view of a typical individual; B, Infraciliature pattern of ventral side; C, Infraciliature of dorsal side. AZM, adoral zone of membranelles; CS, cytostome; CV, contractile vacuole; MA, macronucleus; SK, somatic kinety. Scale bars: A = 200 μm, B = 100 μm.

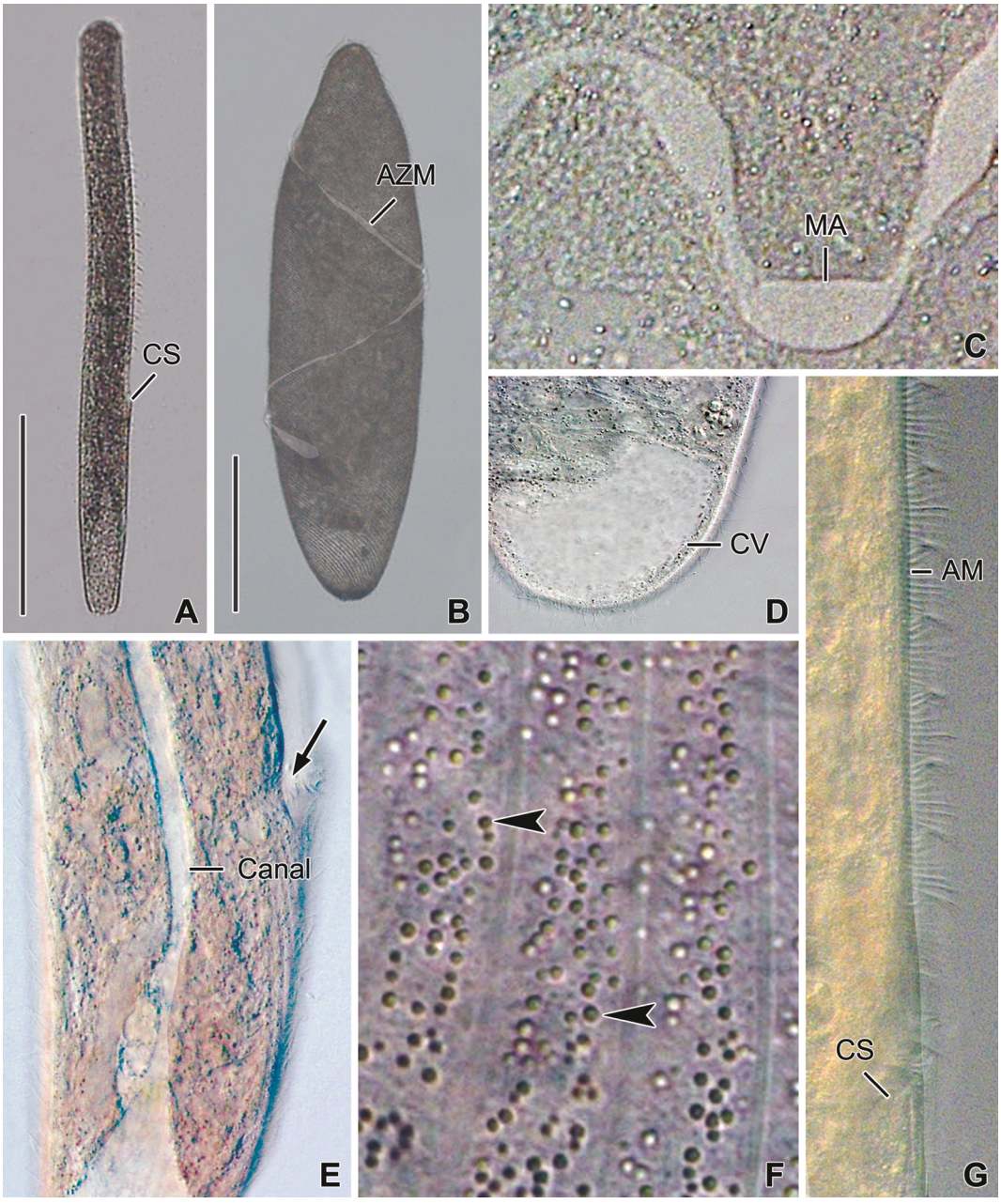

Fig. 2. Microphotographs of *Spirostomum ambiguum* from live specimens (A–G). A, Right side view of a typical specimen; B, Contracted status; C, Moniliform macronuclei; D, Contractile vacuole in posterior end of body; E, Canal to anterior part and cytostome (arrow); F, Arrangement of cortical granules (arrowheads) in between somatic kineties; G, Adoral membranelles with cytostome. AM, adoral membranelles; AZM, adoral zone of membranelles; CS, cytostome; CV, contractile vacuole; MA, macronuclear. Scale bars: A = 200 μm, B = 100 μm.

Distribution. England, India, Jamaica, Japan, Russia, Sweden, USA, and Korea (present study).

Remarks. The Jindo population of *Spirostomum ambiguum* closely resembles the European original population (Müller, 1786) and other European populations (Foissner et al., 1992; Boscaro et al., 2014) with respect to the shape of the macronucleus and ratio of body length to body width, number of cortical granular rows in between somatic kineties, and color of cortical granules (Müller, 1786). However, Jindo population differs from British population in the body length (340–930 μm vs. 1,000–4,000 μm) (Foissner et al., 1992) (Tables 1, 2). The characteristics of Jindo population

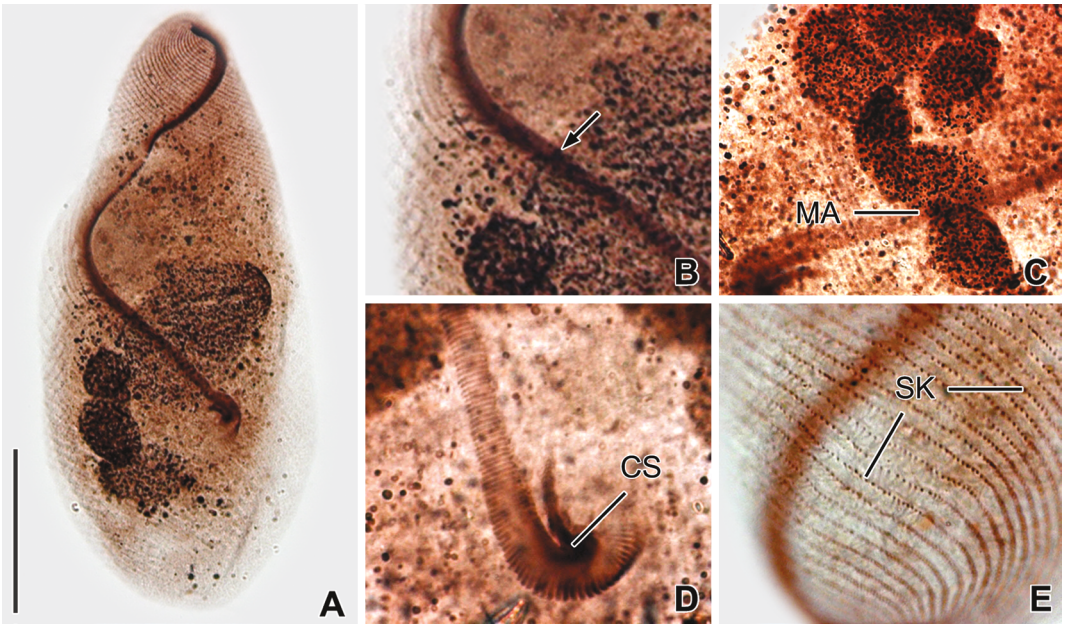

Fig. 3. Microphotographs of *Spirostomum ambiguum* after protargol impregnation (A–E). A, Infraciliature pattern and nuclear apparatus; B, Adoral zone of membranelles (arrow); C, Macronucleus; D, Cytostome; E, Impregnated somatic basal body. CS, cytostome; MA, macronuclear; SK, somatic kinety. Scale bar: A = 100 μm.

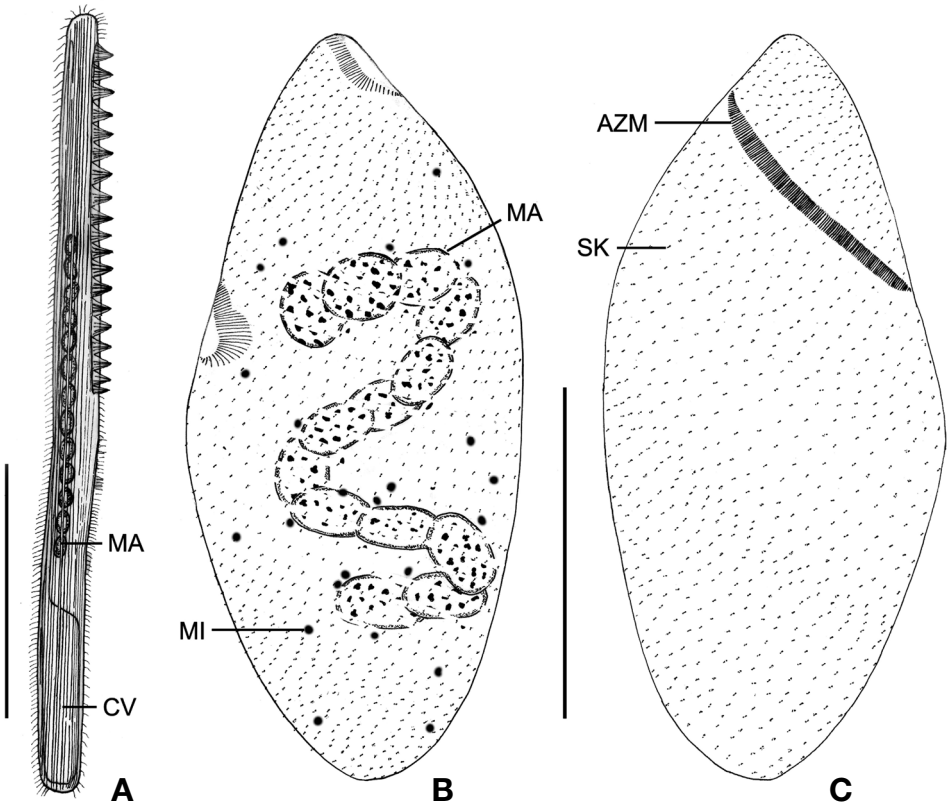

Fig. 4. *Spirostomum minus* from a live specimen (A) and after protargol impregnation (B, C). A, Right side view of a typical individual; B, Infraciliature pattern of ventral side; C, Infraciliature of dorsal side. AZM, adoral zone of membranelles; CV, contractile vacuole; MA, macronucleus; MI, micronucleus; SK, somatic kinety. Scale bars: A = 200 μm, B = 100 μm.

differ from those of Swedish population in the body length (340–930 μm vs. 900–thousands μm), in the ratio of the adoral zone of membranelles to body length (60–80% vs. 50–66%) and the ratio of contractile vacuole to body length (6–17% vs. 10>%) (Boscaro et al., 2014) (Tables 1, 2). The Jindo population of *S. ambiguum* has conspicuously short body length compared to that of other populations in previous investigations (Foissner et al., 1992; Boscaro et al., 2014). *Spirostomum ambiguum* is most similar to *S. minus*. Differences between them are compared in the remarks on *S. minus* in the following section. *Spirostomum ambiguum* is similar to *S. subtilis* but differ in body length/width ratio (10–15 vs. 20–25) and in the number of cortical granular rows in between somatic kineties (4–5 vs. 1) (Boscaro et al., 2014).

***Spirostomum minus* Roux, 1901 (Tables 1, 2, Figs. 4–6)**

Spirostomum ambiguum var. *minor* Roux, 1901: 80.

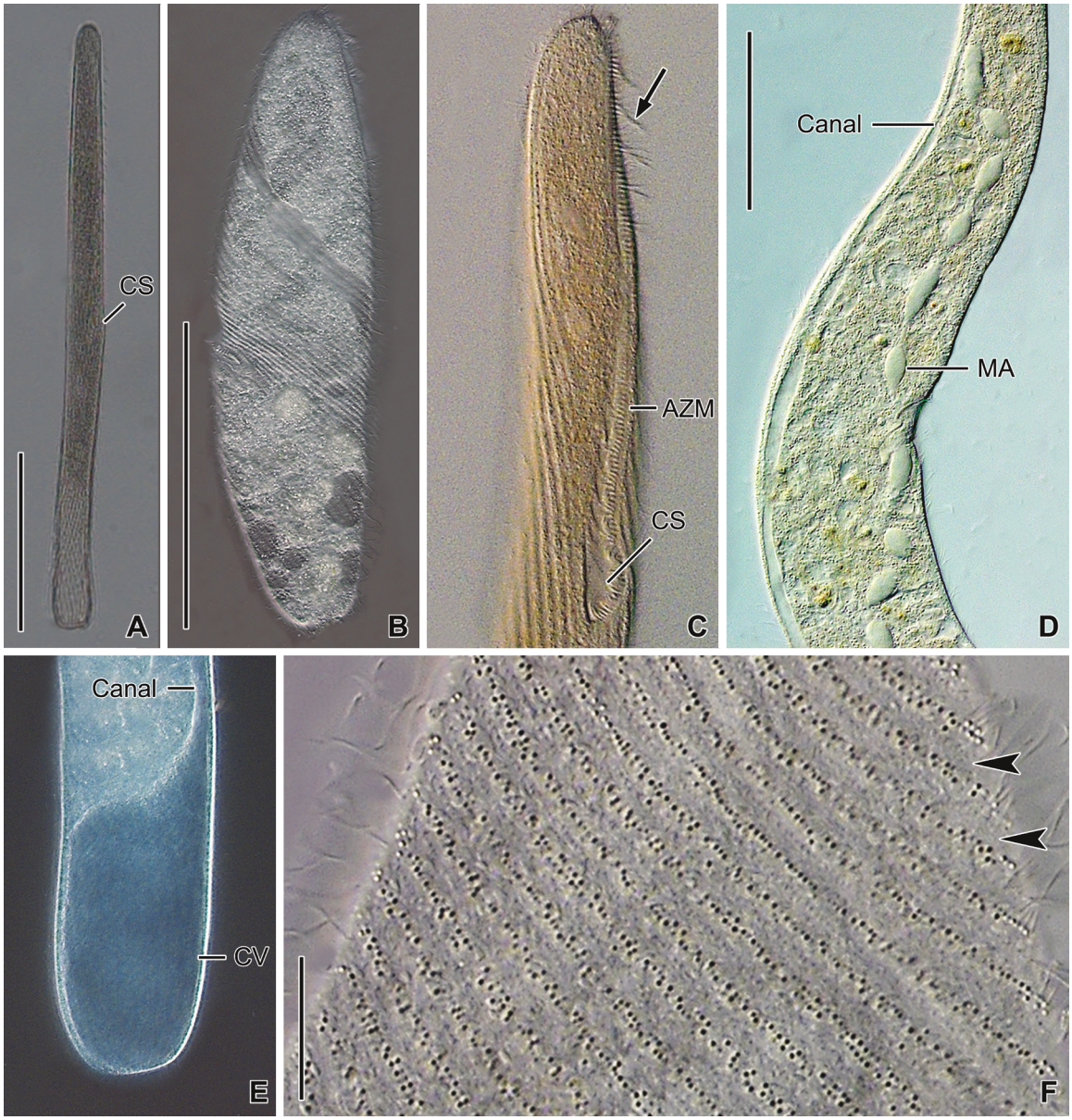

Fig. 5. Microphotographs of *Spirostomum minus* from live specimens (A–F). A, Right side view of a typical specimen; B, Contractile status; C, Adoral zone of membranelles, cytostome and adoral membranelles (arrow); D, Macronucleus and canal to anterior part; E, Contractile vacuole on posterior end; F, Pattern of cortical granule between somatic ciliary rows (arrowheads). AZM, adoral zone of membranelles; CS, cytostome; CV, contractile vacuole; MA, macronuclear. Scale bars: A=200 μm, B=100 μm.

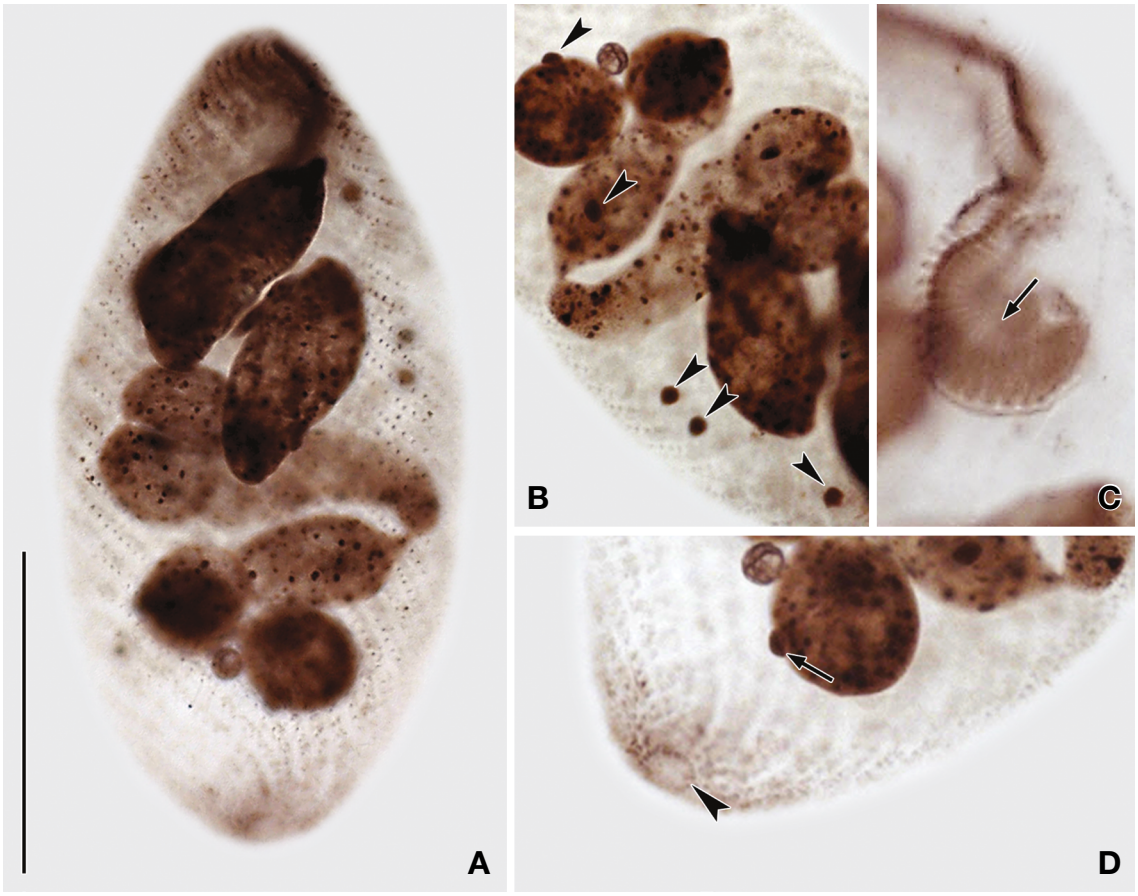

Fig. 6. Microphotographs of *Spirostomum minus* after protargol impregnation (A–D). A, Infraciliature pattern and nuclear apparatus; B, Macronucleus and micronucleus (arrowheads); C, Cytostome (arrow); D, Impregnated somatic basal body, micronucleus (arrow) and cytopyge (arrowhead). Scale bar: A = 100 μm.

Spirostomum intermedium Kahl, 1932: 440.

Spirostomum minus: Kahl, 1932: 440; Boggs, 1965: 603; Repak and Isquith, 1974: 329; Dragesco and Dragesco-Kernéis, 1986: 377; Song and Wilbert, 1989: 145; Foissner et al., 1992: 327; Fernandes and da Silva Neto, 2013: 72; Boscaro et al., 2014: 527.

Diagnosis. Body size on average 630 × 43 μm *in vivo*; long and slender body with truncated posterior end; macronuclei moniliform with 11–16 nodules; 20–37 spherical to oval shaped micronuclei; cortical granules regularly arranged 3–4 rows in between somatic kineties; 20–30 somatic kineties arranged longitudinally; adoral zone of membranelles covered about 40–50% of body length with 120–150 membranelles.

Description. Body size 500–730 × 35–45 μm, approximately 620 × 40 μm *in vivo*. Body flexible, long and slender with rounded anterior and truncated posterior ends, body length to width ratio approximately 14 : 1 (Figs. 4A, 5A), and fusiform body when contracted (Figs. 4B, 5B, 6A). Macronuclei moniliform with 11–16 nodules (Figs. 4A, B, 5D, 6A, B), nodule size approximately 25 × 20 μm in live specimens (Figs. 4A, 5D). Micronuclei spherical to oval shaped scattered in whole body, 20–37 in number, each micronucleus diameter 2.0–2.5 × 1.0–1.5 μm in impregnated specimen (Figs. 4B, 6B). Contractile vacuole located in posterior end, occupied approximately 1/4–1/12 of body length with one long canal that extended to anterior end of dorsal side (Figs. 4A, 5D, E). Cytopyge located at posterior end of dorsal side (Fig. 6D). Cytoplasm colorless. Cortical granules regularly arranged in 3–4 rows between somatic kineties, colorless, approximately 0.4 μm in diameter *in vivo* (Fig. 5F). Movement relatively slow, usually spirally swimming around its body axis. Somatic kineties longitudinally arranged and 20–30 in number, commenced along the apical end to left side of the adoral zone of membranelles and spirally curved when contracted (Figs. 4B, C, 5B, F), somatic ciliary dikinetid approximately 6 μm long. Adoral zone of membranelles oc-

cupied 40–50% of body length and consisted of 120–150 membranelles (Figs. 4A, 5A, C). Cytostome located at proximal end of adoral zone of membranelles, crescent shaped (Figs. 4A, B, 5C, 6C).

Distribution. Brazil, China, England, Germany, India, Madagascar, USA, and Korea (present study).

Remarks. The Jindo population of *Spirostomum minus* closely resembles the original Swiss population (Roux, 1901), British population (Foissner et al., 1992), and Brazilian populations (Fernandes and da Silva Neto, 2013) with respect to ratio of adoral zone membranelles to body length, shape of macronucleus, and ratio of body length to width. However, the Jindo population differs slightly from the Madagascarian population in number of macronucleus nodules (11–16 vs. 24), the German population in ratio of adoral zone of membranelles to body length (40–50% vs. 28–40%), the Brazilian population in body width (35–45 μm vs. 55–115 μm) and color of cortical granules (colorless vs. pale brown), and the European population in number of micronuclei (20–37 vs. up to 20) (Dragesco and Dragesco-Kernéis, 1986; Song and Wilbert, 1989; Fernandes and da Silva Neto, 2013; Boscaro et al., 2014) (Table 2). Typical *Spirostomum minus* and *S. ambiguum* are very similar but different with respect to body length (300–800 μm vs. 1,000–4,000 μm) and ratio of adoral zone membranelles to body length (40–50% vs. 65–70%) (Foissner et al., 1992) (Tables 1, 2).

ACKNOWLEDGMENTS

We appreciate two anonymous reviewers who gave comments and suggestions to improve the manuscript. This study was supported by the grants from the National Institute of Ecology, funded by the Ministry of Environment of the Republic of Korea and the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2016R1D1A2B 03933285, 2015R1D1A09058911).

REFERENCES

Boggs N Jr, 1965. Comparative studies on *Spirostomum*: silver impregnation of three species. The Journal of Protozoology, 12:603-606. https://doi.org/10.1111/j.1550-7408.1965.tb03261.x

Boscaro V, Carducci D, Barbieri G, Senra MVX, Andreoli I, Erra F, Petroni G, Verni F, Fokin SI, 2014. Focusing on genera to improve species identification: revised systematics of the ciliate *Spirostomum*. Protist, 165:527-541. https://doi.org/10.1016/j.protis.2014.05.004

Claparède É, Lachmann J, 1858. Etudes sur les Infusoires et les Rhizopodes. Mémoires de l'Institut National Genevois, 5:1-260.

Curds CR, Gates MA, Roberts DML, 1983. British and other freshwater ciliated protozoa. part II. Ciliophora: Kinetofragminophora. The Linnean Society of London and The Estuarine and Brackish-Water Sciences Association, Cambridge, pp. 1-414.

Dragesco J, Dragesco-Kernéis A, 1986. Ciliés libres de l'Afrique intertropicale: introduction á la connaissance et á l'étude des ciliés. Faune Tropicale, 26:1-559.

Ehrenberg CG, 1833. Dritter Beitrag zur Erkenntniss groβer Organisation in der Richtung des kleinsten Raumes. Abhandlungen der Akademie der Wissenschaften der DDR, Berlin, pp. 145-336.

Ehrenberg CG, 1835. Zusätze zur Erkenntniß großer organischer Ausbildung in den kleinsten thierischen Organismen. Abhandlungen der Akademie der Wissenschaften der DDR, Berlin, pp. 151-180.

Ehrenberg CG, 1838. Die Infusionsthierchen als vollkommene Organismen. Ein Blick in das tiefere organische Leben der Natur. Verlag von Leopold Voss, Leipzig, pp. 1-547.

Fernandes NM, da Silva Neto ID, 2013. Morphology and 18S rDNA gene sequence of *Spirostomum minus* and *Spirostomum teres* (Ciliophora: Heterotrichea) from Rio de Janeiro, Brazil. Zoologia, 30:72-79. https://doi.org/10.1590/S1984-46702013000100009

Foissner W, Berger H, Kohmann F, 1992. Taxonomische und Ökologische Revision der Ciliaten des Saprobiensystems, Band II. Peritrichia, Heterotrichida, Odontostomatida. Informationsberichte des Bayerichen Landesamtes für Wasserwirtschaft, 5/92:1-502.

Jang SW, Kwon CB, Shin MK, 2012. First records of two *Spirostomum* ciliates (Heterotrichea: Heterotrichida: Spirostomidae) from Korea. Animal Systematics, Evolution and Diversity, 28:29-35. https://doi.org/10.5635/ASED.2012.28.1.029

Kahl A, 1932. Urtiere oder Protozoa. I: Wimpertiere oder Ciliata (Infusoria). 3. Spirotricha. Die Tierwelt Deutschlands, 25:399-650.

Li L, Huang J, Song W, Shin MK, Al-Rasheid KAS, Berger H, 2010. *Apogastrostyla rigescens* (Kahl, 1932) gen. nov., comb. nov. (Ciliophora, Hypotricha): morphology, notes on cell division, SSU rRNA gene sequence data, and neotypification. Acta Protozoologica, 49:195-212.

Lynn DH, 2008. The ciliated protozoa: characterization, classification, and guide to the literature. 3rd ed. Springer Publishers, New York, pp. 1-605.

Müller OF, 1786. Animalcula infusoria fluvia tilia et marina/que detexit, systematice descripsit et ad vivum delineari curavit Otho Fridericus Müller; Opus hoc posthumum, quod cum tabulis Aeneis L. in lucem tradit vidua ejus nobilissima cura Othonis Fabricii. N. Mölleri, Hauniae, pp. 1-605.

Repak AJ, Isquith IR, 1974. The systematics of the genus *Spirostomum* Ehrenberg, 1838. Acta Protozoologica, 12:325-

333.

Roux J, 1901. Faune infusorienne des eaux stagnantes des environs de Genève. Mémoires de l'Institut National Genevois, 19:1-149.

Shazib SUA, Vd'ačný P, Kim JH, Jang SW, Shin MK, 2014. Phylogenetic relationships of the ciliate class Heterotrichea (Protista, Ciliophora, Postciliodesmatophora) inferred from multiple molecular markers and multifaceted analysis strategy. Molecular Phylogenetics and Evolution, 78:118-135. https://doi.org/10.1016/j.ympev.2014.05.012

Shigenaka Y, 1959. A new marine ciliate, *Spirostomum yagiui* n.sp. Zoological Magazine, 68:368-372.

Song W, Wilbert N, 1989. Taxonomische Untersuchungen an Aufwuchsciliaten (Protozoa, Ciliophora) im Poppelsdorfer Weiher, Bonn. Lauterbornia, 3:2-221.

Stein F, 1867. Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. II Abtheilung. 1) Darstellung der neuesten Forschungsergebnisse über Bau, Fortpflanzung und Entwickelung der Infusionsthiere. 2) Naturgeschichte der heterotrichen Infusorien. Wilhelm Engelmann, Leipzig, pp. 1-355.

Wilbert N, 1975. Eine verbesserte Technik der Protargolimprägnation für Ciliaten. Mikrokosmos, 64:171-179.

Received November 7, 2016
Revised November 24, 2016
Accepted November 25, 2016